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A CYTOGENETIC STUDY DEALING WITH THE TRANSFER OF GENES FROM TRITICUM TIMOPHEEVI TO COMMON WHEAT BY BACKCROSSING[1]

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INTRODUCTION

Correlated genetic and cytological studies have only infrequently accompanied the transfer of desirable genes from one species to another by means of repeated backcrossing. This paper describes such a study of the hybrid between *Triticum vulgare*[3] Vill. ($n=21$) and *Triticum timopheevi* Zhuk. ($n=14$).

REVIEW OF LITERATURE

Triticum timopheevi, a species endemic to the foothills of the Caucasian mountains, was first described as a variety of *T. dicoccum* Schrank. var. *dicoccoides* Körn. by Zhukovskii (*37*)[4] in 1923. In 1928 it was elevated to specific rank under the name *T. timopheevi* Zhuk. (*38*). Zhukovskii (*38*) and Sando (*29*) have given detailed botanical descriptions of the species.

The new species soon attracted considerable attention, not only because of its high disease resistance (*5*, *13*, *17*, *26*, *28*, *31*) but also because of the report by Kihara and Lilienfeld (*19*) that it possessed a genome, designated *GG*, not previously known to occur in the wheat genus. Kostoff (*20*), however, regarded the second genome of *Triticum timopheevi* as only a modification of the *BB* genome of the emmer wheats and designated it $\beta\beta$. Love (*24*) preferred to assign no distinctive formula for the species, stating that it differed from the other 28-chromosome wheats in degree of divergence only. Svetozarova (*33*) has since shown that 14 bivalents occur in the hybrid of *T. timopheevi* with *T. armeniacum* (Jacubz.) Makush. ($n=14$); presumably these species are more closely related to each other than to other 28-chromosome wheats.

Triticum timopheevi has been demonstrated to be highly sterile in crosses with all other species of wheat including *T. armeniacum* (*17*,

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[3] The priority of *Triticum aestivum* L. for the name of the species more commonly known as *T. vulgare* is recognized. Because the latter name is in general use by agronomists the world over, preference is given to this form.

[4] Italic numbers in parentheses refer to Literature Cited, p. 62.

19, 20, 28, 31, 33). Despite this sterility some of its disease resistance was transferred to common wheat by Pridham (*28*). He was able to select fertile lines resistant to stem and leaf rusts and of fair agronomic type by the F_5 generation. Shands (*31*) obtained fertile stem- and leaf-rust-resistant lines with 21 pairs of chromosomes following one backcross to *T. vulgare* (the male parent) and 5 generations of selfing.

A general review of the cytology of interspecific wheat hybrids has been given by Aase (*1*). Worthy of special mention was the demonstration by Kihara (*18*) that the parental chromosome numbers were regained during several generations of self-pollination of the moderately fertile pentaploid wheat hybrids. According to Kihara, two series were formed, an ascending series from which 42-chromosome plants resulted eventually, and a descending series which resulted in 28-chromosome plants. The ascending series was characterized by 15 to 21 pairs of chromosomes with the sum of the bivalents and univalents numerically equal to 21. The descending series was characterized by 14 pairs plus 1 to 6 univalents, the univalents gradually being lost until a condition of 14 pairs was reached. *Vulgare*-like plants (ascending series) were much less frequent than emmer-like plants (descending series); this observation has since been voluminously confirmed (*11, 12*). Certain *Nicotiana* hybrids also conform to Kihara's scheme, as shown by Lammerts (*21*). Despite the return to parental chromosome numbers meiotic instability may persist for many generations in derivatives from pentaploid wheat hybrids (*23, 25, 30*).

In interspecific hybrids a greater proportion of female gametes than male gametes are usually functional (*14, 21, 34, 36*), although exceptions occur, as in certain *Gossypium* hybrids (*8, 9, 10*). When pentaploid wheat hybrids are backcrossed to the common wheat parent, there is severe selection against male gametes with intermediate chromosome numbers, but female gametes with 14 to 21 chromosomes function about equally frequently (*34, 36*). Thus, in plant hybrids, a striking difference frequently has been noted in the progenies derived from reciprocal backcrosses.

The backcross method has been used by several investigators to increase fruitfulness in the progenies of interspecific hybrids. Three or four backcrosses have usually been sufficient to restore apparently complete fertility in several highly sterile hybrids (*4, 8, 9, 10, 14, 15, 32*).

There is rather general agreement concerning the number of backcrosses necessary to recover the type of the recurrent parent. Harland and Atteck (*10*), working with interspecific *Gossypium* hybrids, reported that "generally speaking from the results of all the backcrossing experiments, it appears to be unnecessary to carry the backcrossing beyond the fourth or at most the fifth backcross, as the heterozygote is by then stabilized on the new genetical background." Holmes (*16*) reached essentially the same conclusion in work with interspecific tobacco hybrids. The most extensive investigations of intraspecific backcrosses are those of Briggs (*3*), whose work with small grains indicated that after three or four successive backcrosses the derivative populations had become so nearly like the recurrent parent that selection for characters other than the one being trans-

ferred was not effective. Six backcrosses were regarded as necessary, however, to recover the recurrent parent in close detail.

MATERIAL AND METHODS

The strain of *Triticum timopheevi* used in this investigation was brought to the United States by Dickson [5] as P. I. [6] 94761. An unnamed spring wheat selection out of the hybrid Illinois No. 1 × Chinese, designated as 2666A2–2–15–6–3 by the Wisconsin Agricultural Experiment Station, was used as the common wheat parent because it was known to function better than several other varieties in hybrids with *T. timopheevi*. The parents will hereafter be designated *vulgare* and *timopheevi*.

In making crosses, spikelets at the two or three top and bottom nodes of the spike, and all but the first two florets of the remaining spikelets, were removed. The remaining florets were emasculated, protected with a glassine bag, and pollinated 1 to 3 days later.

Seed set was determined by calculating the percentage of pollinated florets or first and second florets of central spikelets on open-pollinated or selfed spikes that produced seeds. Self-pollination was enforced by covering some spikes with glassine bags from 2 or 3 days before anthesis to maturity. Backcross fertility was calculated from not less than 40 florets and self-fertility or open-pollinated fertility from not less than 100 florets per plant.

Using *V* and *T* to represent *vulgare* and *timopheevi*, respectively, the following will outline the symbols used to designate hybrids, backcrosses, and the selfed generations therefrom. The seed parent is written first and the pollen parent second throughout.

Origin:	*Designation*
$V \times T$	F_1.
F_1 hybrid allowed to open-pollinate	F_2 (o. p.).[7]
F_1 hybrid protected from outcrossing	F_2.
$V \times (V \times T)$	V(VT).
$(V \times T) \times T$	(VT)T.
$(V \times T) \times V$	BC^1.
$[(V \times T) \times V] \times V$	BC^2.
$V \times [(V \times T) \times V]$	RBC^2.[8]
$V \times (V \times [(V \times T) \times V])$	RBC^3.
BC^1 protected from outcrossing	BC^1F_2.
BC^1 allowed to open-pollinate	BC^1F_2 (o. p.).

Unless indicated by (o. p.), pollination was controlled in all cases.

All cytological observations were made from acetocarmine smear preparations. Twenty-five pollen mother cells (P. M. C.) at metaphase of the first meiotic division were analyzed for pairing from each of the parental plants, from the F_1, from V (VT) plants, and from 40 BC^1 plants. In the remaining BC^1 plants and in all other generations, only 10 P. M. C. per plant were analyzed. One-hundred microspores still in the form of quartets were examined in each plant to determine the

[5] DICKSON, J. G. CEREAL DISEASES STUDIES IN EUROPE AND ASIA. 1930. [Unpublished manuscript in the University of Wisconsin library.]

[6] P. I. refers to the accession number of the Division of Plant Exploration and Introduction, United States Department of Agriculture (formerly Foreign Plant Introduction).

[7] (o. p.) stands for open-pollinated.

[8] R stands for reciprocal. Superscript 2 is used to emphasize that this generation received the same number of backcrosses to *vulgare* as BC^2, although only one of the two backcrosses was in the reciprocal direction

frequency of micronuclei. Microcytes were tabulated separately, but because of their sporadic occurrence were later included as micronuclei.

The amount of good pollen was determined from anthers collected at anthesis, fixed in Carnoy's solution, and stained with acetocarmine. Only those pollen grains were considered "good" which possessed two gametes and a vegetative nucleus, the condition characteristic of the pollen of both parents. Estimates of the percentage of good pollen were based upon the examination of 200 pollen grains per plant.

In order to obtain an unbiased sample of pollen mother cells, quartets of microspores, or pollen grains, all units were counted within random strips from one side of the slide to the other.

An attempt was made to obtain a random sample from each backcross generation for morphological and cytological study by determining by random methods before planting the plants to be studied. Not less than 20 hybrid plants were used as parents to provide as wide a base as possible for each backcross generation. Many families in excess of those studied cytologically or morphologically were grown in separate nurseries, and were useful for studies of disease resistance and for additional observations upon morphological characteristics.

THE PARENTAL SPECIES

Some contrasting characters of the common wheat parent and *timopheevi* are given in table 1. Spikes are illustrated in figure 1, *A* and *E*. It will subsequently be shown that both parents are fertile and cytologically regular.

TABLE 1.—*Expressions of certain morphological characters observed in vulgare, timopheevi, and in the F_1 hybrid*

Character	Expression of—		
	Vulgare	Timopheevi	F_1 hybrid
Diseases:			
Stem rust	Susceptible	Resistant	Nearly like *timopheevi*.
Bunt	Susceptible	do	Susceptible.
Mildew	Moderately susceptible.	do	
Leaf rust	Fairly resistant	do	Moderately susceptible.
Glume characters:			
Adherence to kernel	Easily detached	Attached tightly	Like *timopheevi*.
Keel	Inconspicuous	Prominent	Do.
Shoulder	Nearly wanting	Narrow with pointed tooth.	Do.
Tip	Beak 5 to 10 millimeters, increasing toward apex of spike.	Beak 5 millimeters or less in length.	Do.
Pubescence	Absent	Long, dense	Intermediate in length and density.
Other characters:			
Lemma	Awned	Awned	Awned.
Rachis articulation	Tough	Moderately fragile	Like *timopheevi*.
Adherence of spikelet to rachis.	Easily detached	Tenacious	Do.
Leaf pubescence	Absent	Long, dense	Intermediate in length and density.
Ciliation of leaf sheath margin.	do	do	Do.
Spike density [1]	5.3 millimeters	2.9 millimeters	4.4 millimeters.
Maturity	Midseason	Late	Very late.
Kernel type	Ovate, red	Long, slender, light red	Very short, intermediate red.
Vigor	Vigorous	Vigorous	Very vigorous.
Straw	Thick	Slender	Intermediate.

[1] Mean length of the 10 central internodes of the spike.

FIGURE 1.—Spikes: *A*, *vulgare* parent; *B*, V (VT); *C*, *vulgare* × *timopheevi* F_1; *D*, *timopheevi* × *vulgare* F_1; *E*, *timopheevi* parent.

THE F_1 GENERATION

Of 375 florets of *vulgare* that were pollinated with pollen of *timopheevi*, 75 percent set seed. This was comparable to the success of intervarietal crosses in *vulgare* made at the same time. The F_1 kernels

were shorter and smaller than selfed kernels of *vulgare*, but had plump endosperms; 89 percent germinated. The reciprocal cross gave a slightly higher seed set. These kernels were badly shrunken, however, and only 4 of 79 germinated. The F_1 plants of the reciprocal crosses were indistinguishable in appearance (fig. 1, *C* and *D*), cytological behavior, and fertility.

The F_1 hybrids were similar to *timopheevi* in some characters, but in most others they were intermediate between the parents (table 1). Except for reaction to bunt, no character of the *vulgare* parent appeared to be governed by dominant genes. The F_1 hybrids were large, sturdy, and tillered profusely.

The 11 F_1 plants examined cytologically each had 35 chromosomes (fig. 2, *C*). Microsporogenesis was similar to that observed by Kostoff (20), Love (24), and Pathak (27), and was in sharp contrast to the regular meiotic behavior of the parents (fig. 2, *A* and *B*, and table 2). The variability in pairing is readily noted from the range in the numbers of each type of association of chromosomes and also by the 42 different combinations of univalents, bivalents, trivalents, and multiple associations. When open and closed [9] bivalents were considered to be different types of association, the numbers of associations exceeded 250 in the 275 pollen mother cells examined.

TABLE 2.—*Meiotic behavior of the parental species, the F_1 hybrid, and of the backcross with the F_1 as the pollen parent* [1]

Cytological character	Vulgare	Timopheevi	F_1 hybrid	V(VT)
Number of plants examined cytologically	11	8	11	11
2*n* chromosome number	42	28	35	35
Bivalents:				
Average	20.86	14.00	8.29	9.03
Range	19–21	0	4–13	5–14
Closed bivalents:				
Average	19.32	12.74	3.22	3.69
Range	14–21	10–14	0–7	1–7
Open bivalents:				
Average	1.54	1.26	5.07	5.34
Range	0–7	0–4	1–9	1–9
Univalents:				
Average	0.16	0	14.79	13.89
Range	0–4	0	7–21	7–21
Trivalents:				
Average	0.01	0	1.04	0.89
Range	0–1	0	0–4	0–4
Quadrivalents:				
Average	0.01	0	0.13	0.13
Range	0–1	0	0–1	0–1
Quinquevalents:				
Average	0	0	0.01	0.01
Range	0	0	0–1	0–1
Number of different pairing arrangements	4	1	42	40
Number of micronuclei per quartet:				
Average	0.15	0.04	4.98	4.89
Range	0–5	0–5	0–12	0–13
Percent quartets with no micronuclei	92	98	1.3	1.0
Percent good pollen	84	94	1.7	0.8
Seed set:				
Open-pollinated	94	97	0.6	0.1
Backcross			5.35	6.52

[1] See Materials and Methods for the number of observations made per individual plant.

[9] Open bivalents have one or more chiasmata confined to a single arm, abbreviated IIo hereafter. Closed bivalents have one or more chiasmata in each arm, giving closed configurations, abbreviated IIc. Univalents and trivalents are abbreviated I and III respectively.

Observations on serial sections of four megaspore mother cells indicated that the chromosome behavior was similar in both megasporogenesis and microsporogenesis. This agrees with the results of Watkins (35) based upon extensive studies of the hybrid *Triticum vulgare* $\times$ *T. turgidum*.

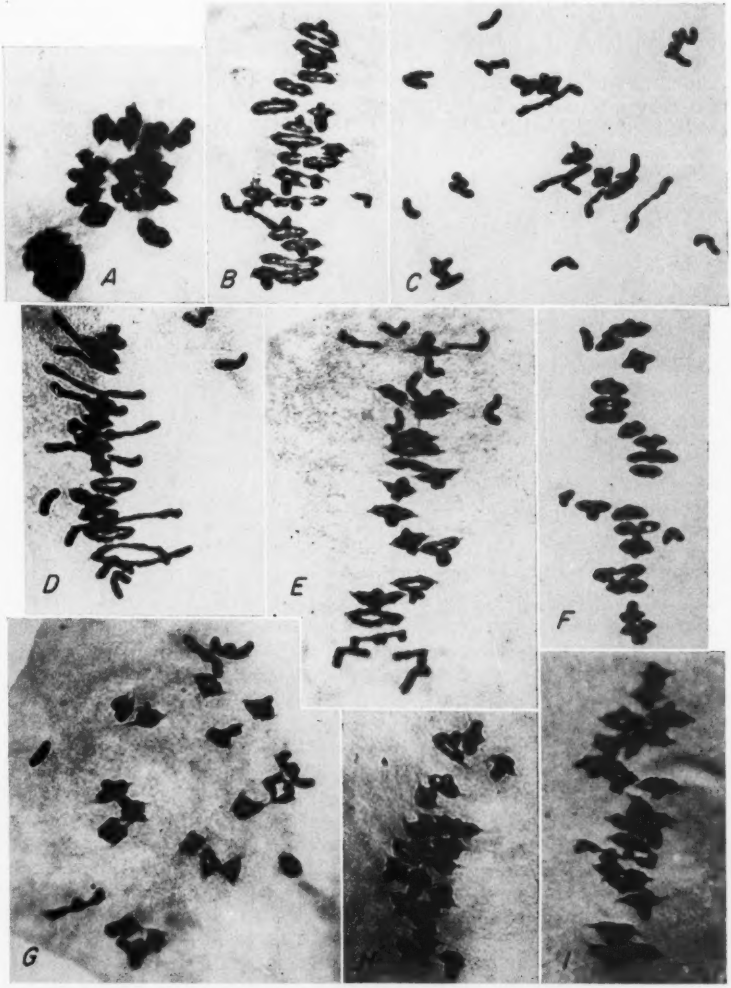

FIGURE 2.—First meiotic metaphases: *A*, 14 IIe in *timopheevi*; *B*, 19 IIe and 2 IIo in *vulgare*, *C*, F_1 hybrid showing 5 IIe, 3 IIo, 16 I, and 1 III; *D*, BC^1 P. M. C. with 10 IIe, 5 IIo, 5 I, and 2 III; *E*, BC^2 P. M. C. with 14 IIe, 3 IIo, and 6 I; *F*, BC^3 P. M. C. with 18 IIe and 3 I; *G*, BC^4 P. M. C. with 17 IIe, 4 IIo, and 1 I; *H*, RBC^3 P. M. C. with 21 IIe; *I*, BC^2F_4 P. M. C. with 20 IIe and 1 IIo.

Variability in pairing in the hybrid, known autosyndesis in *vulgare*, and lack of recognizable individuality of chromosomes complicate the estimation of homology between the chromosomes of the two species. The occurrence of an average of 3 closed pairs suggests that some chromosomes may be almost completely homologous. Since up to 13 (open and closed) pairs were observed, and if autosyndesis was not occurring, it might be assumed that nearly all of the chromosomes of *timopheevi* have segments homologous to portions of *vulgare* chromosomes.

Incomplete pairing of chromosomes in species hybrids may be determined by chromosome homology, environment, or possibly by genes. An analysis of meiotic behavior in F_1 hybrid plants revealed no significant

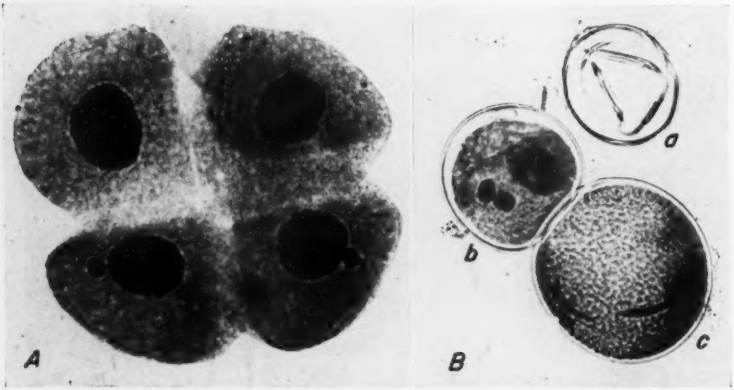

FIGURE 3.—*A*, Quartet of microspores with a single micronucleus in each of two microspores. *B*, Pollen grains: *a*, Aborted and, *b*, retarded pollen grains; *c*, a "good" pollen grain with two male gametes and a vegetative nucleus.

differences in pairing between material collected at 11 different dates from plants grown in both the greenhouse and the field at Madison, Wis., in the period 1941 to 1943. This study has been reported in detail elsewhere.[10] Because the 11 dates included a wide range of conditions, it was concluded that environmental influences did not contribute significantly to the differences in meiotic behavior observed. Since there is also no evidence that gene action is responsible for the incomplete pairing either in the F_1 or later generations, incomplete homology of chromosomes appears the most reasonable explanation. If chromosomes of *timopheevi* varied in their homologies with chromosomes of *vulgare*, it would be expected that genes in the more homologous chromosomes would be easier to transfer to common wheat.

Irregular pairing at meiosis resulted in large numbers of laggards which frequently remained in the cytoplasm as micronuclei (table 2, fig. 3, *A*).

About 1 percent of "good" pollen was found in anthers of F_1 hybrids (table 2, fig. 3, *B*, *c*). The remainder of the pollen was mostly com-

[10] ALLARD, R. W. A CYTOGENETIC STUDY OF THE EFFECT OF BACKCROSSING TO COMMON WHEAT IN A HYBRID BETWEEN TRITICUM VULGARE VILL. AND TRITICUM TIMOPHEEVI ZHUK. 1946. [Unpublished Ph. D. thesis. Copy on file University of Wisconsin library, Madison.]

pletely empty (fig. 3, *B*, *a*). A small percentage of grains had some cytoplasm but only one or sometimes two or three nuclei (fig. 3, *B*, *b*).

A seed set of 5.4 percent was obtained when pollen of *vulgare* was used on the F_1. Under open pollination approximately 0.6 percent of the florets set seed. When isolated in the greenhouse or protected from outcrosses in the field with glassine bags only 5 kernels were obtained from more than 10,000 florets, a seed set of less than 0.05 percent. Plants obtained from the kernels above are discussed under BC_1F_2 (o. p.), and F_2 generations, respectively. Kernels obtained from the backcross V(VT) are considered in the following section.

THE BACKCROSS V(VT)

The backcrosses were made in a greenhouse in which no *timopheevi* was growing, excluding the possibility that the seeds resulted from accidental crossing with that species.

The pollination was performed by breaking open the leathery anthers of the F_1 and brushing them upon the stigmas of *vulgare*. This laborious process resulted in 32 kernels from 1,370 florets. Three of these kernels resembled *vulgare* and produced plants of that type, probably the result of accidental selfing or outcrossing. The remote possibility exists, however, that pollen grains from F_1 plants which had exactly the monoploid complement of *vulgare* effected pollination. Twenty-nine of the 32 kernels resembled F_1 kernels in appearance. Of these, 25 germinated and produced mature plants which were morphologically indistinguishable from each other and from plants of the F_1 generation (fig. 1, *B*).

Eleven of the 25 backcrossed plants were examined cytologically and all were found to have 35 chromosomes (table 2). Conjugation was similar to that in F_1 plants and they were also similar in frequency of micronuclei, proportion of good pollen, and fertility (table 2). However, there was a significantly greater number of pairs and fewer univalents in the backcrossed population than in the F_1 population.[11] This provides evidence that crossing-over had occurred between some chromosomes and that functional pollen of the F_1 on the average possessed slightly more chromatin homologous to that of common wheat than did pollen of *timopheevi*. Alteration of chromosomes carried by functional F_1 pollen was slight, however, and backcrosses in this direction were discontinued when it became evident that only slow progress in transferring genes of *timopheevi* to common wheat could be expected.

THE BC^1 GENERATION AND ITS DERIVATIVES

MORPHOLOGICAL REGRESSION TOWARD THE RECURRENT PARENT

The pollination of 5,425 flowers of F_1 plants with pollen of *vulgare* resulted in 290 kernels, of which 119 germinated and produced mature plants. In contrast to the backcross V(VT), a wide range of segregation occurred, all plants, however, varying about the *vulgare* type (fig. 4, *B–D*). With further backcrosses to *vulgare* the population assumed an even more *vulgare*-like appearance (fig. 5, *B–D*). A random group of several hundred plants from backcross and backcross derivative generations was classified for the morphological characters

[11] See footnote 10, p. 40.

listed in table 1. The plants were divided into 5 classes varying from like *vulgare* to like *timopheevi*. A detailed account of this classification has been presented elsewhere.[12]

FIGURE 4.—Spikes: *A*, *vulgare*; *B–D*, some spike types observed in the BC_1 generation; *E*, *timopheevi*.

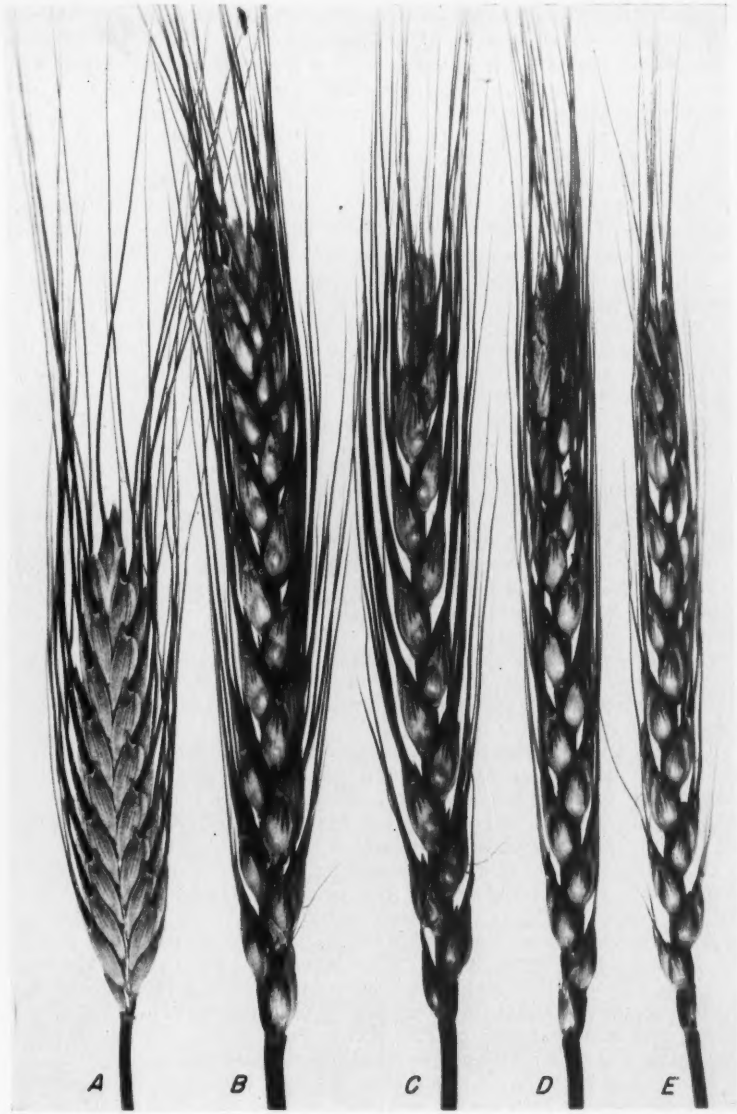

FIGURE 5.—Spikes: *A*, *vulgare*; *B–D*, some spike types observed in the BC^4 generation; *E*, *timopheevi*.

The results indicated that different characters disappeared from the population or lost their *timopheevi*-like expression at different rates with successive backcrosses. For example, the *timopheevi*-like shape of the glume beak, the keel on the glume, and the glume shoulder

appeared to be retained longer than other characters studied. Though all 3 are glume characters, they appeared independently in different plants and hence must be governed by different genes. More than 90 percent of the BC^1 plants resembled *timopheevi* more closely than *vulgare* in these characters. Even after 3 backcrosses the glume keels of 54 percent of the population resembled those of *timopheevi;* and the 2 other characters, shape of glume beak and shoulder, were *timopheevi*-like in 44 and 19 percent of the BC^3 population, respectively. After 4 backcrosses many plants retained a *timopheevi*-like expression of these characters. The 3 glume characters occasionally became fixed in *vulgare*-like lines by selfing backcrossed plants.

A group of characters somewhat less strongly retained in backcrossed populations were pubescence on the laminae, glumes, and cilias on the margins of the leaf sheaths. These characters appeared independently in different plants. Although 38 to 52 percent of the BC^1 plants possessed pubescence of intermediate length and density, three backcrosses served to eliminate pubescence almost completely. Pubescence became fixed in some lines following selfing, but much less frequently than the glume characters discussed earlier.

Unlike the six previously mentioned characters which are economically unimportant, the tight envelopment of the caryopsis by the glumes, lemma, and palea, the strong adherence of the spikelet to the rachis, and the easy articulation of the rachis are characters of *timopheevi* which are very undesirable economically because they contribute to preharvest shattering and threshing difficulties. These characters appeared in nearly all BC^1 plants but usually with an expression intermediate between the parental species. After three backcrosses few if any traces of difficult threshing remained, and four backcrosses eliminated these characteristics completely. Rarely did these characters become fixed in lines derived by selfing early backcross generations.

Classification of plants of backcrossed populations for kernel type, maturity, leaf and straw type, and spike characters proved difficult because of numerous intergrades. However, the *timopheevi*-like expression of these characters usually failed to survive more than one or rarely two backcrosses to *vulgare*.

A striking difference in the rate of regression toward the recurrent parent was noted between the BC^2 and RBC^2 populations. When BC^1 plants were the pollen parents almost all *timopheevi* characters were screened out so that the RBC^2 population much more closely resembled the recurrent parent than when BC^1 plants were the seed parents. The RBC^3 population also more closely resembled the recurrent parent than the comparable BC^3 population. Thus, the functional male gametes of BC^1 plants were genetically much more like *vulgare* than were the functional female gametes.

In summary, it may be said that a single backcross to *vulgare* as the male parent resulted in a highly diverse population which resembled that species grossly, but retained many characters of *timopheevi* in a form at least intermediate between the parents. During two more backcrosses the characters of *timopheevi* were lost or diluted, some more rapidly and completely than others, and the backcross population became much more uniform. However, after four back-

[12] See footnote 10, p. 40.

crosses the population still varied rather widely about the type of the recurrent parent even though few evidences of *timopheevi* parentage remained. The uniformity expected on the basis of published accounts (*9*, *10*, *15*) was lacking, although a few individuals nearly identical with the recurrent parent were found. Backcrosses with BC^1 and RBC^2 plants as pollen parents resulted, however, in a much more rapid return to the type of *vulgare*. Studies of the transmission of disease reaction, which is economically more important than the characters discussed above, are reported in the two following sections.

RUST STUDIES

The reaction of several generations to stem rust caused by *Puccinia graminis tritici* Eriks. and Henn. was determined in the field in the summers of 1942, 1943, and 1944. Epiphytotics of the disease were induced by inoculation early in the season of guard rows of a susceptible variety with races 19 and 56. Other races were known to have occurred naturally. The most important of these races was 15B, which appeared in the nursery in 1943. Race 15B was identified by Dr. E. C. Stakman, of the University of Minnesota, who also furnished the inoculum of races 19 and 56. Variety 2666A2–2–15–6–3, the *vulgare* parent, proved quite resistant to leaf rust caused by *Puccinia triticina* Eriks. and none of the backcrossed progeny showed more than a light infection by this disease in any of the several years they were under observation. Some of the backcrossed plants and their derivatives possessed a very high order of resistance to leaf rust that apparently came from the *timopheevi* parent.

The "host response" to stem or leaf rust was determined by comparison with the standard scale for estimating rust precentages adopted by the Division of Cereal Crops and Diseases, United States Department of Agriculture (*22*). The severity of infection expressed as a percentage figure was also recorded for each plant. Both scales were later used in determining degree of resistance.

In each season a satisfactory epiphytotic of stem rust developed. Susceptible varieties such as the *vulgare* parent became infected early in the season. Soon after heading, the severity of infection reached 90 percent on most plants with pustules of the very or completely susceptible type, and only shriveled caryopses were produced. *Timopheevi* had an apparently immune type of "host response" with pustules appearing only rarely and then late in the season. In 1943 occasional plants of *timopheevi* had up to 1 percent of rust, possibly because of the presence of race 15B. The F_1 appeared to be highly resistant until heading time, when a few pustules were observed. These increased until 5 percent severity of infection was reached near maturity in 1942 and 1944 and 10 percent in 1943. The stem rust resistance of *timopheevi* is thus not completely dominant to the susceptibility of the *vulgare* parent.

In the 1943 season 70 BC^1 plants were tested for stem rust reaction. They appeared to fall into three groups on the basis of host response and severity of infection: (1) Plants which were entirely free from rust or developed a few pustules of the apparently immune type late in the season; (2) plants with a fairly resistant host response and of variable severity of infection, mainly from 1 to 5 percent, but occasionally reaching 40 percent in late-maturing plants; and (3) plants as suscep-

tible as the common wheat parent. The 70 BC^1 plants fell into the resistant, intermediate, and susceptible groups in the proportion of 28:31:11, respectively. Simultaneously 169 BC^2 individuals from 22 different BC^1 plants and 43 RBC^2 individuals from 7 BC^1 plants were tested for stem rust reaction. The backcrosses had been made in the greenhouse so that the rust reaction of the BC^1 parents was unknown. The proportion of resistant, intermediate, and susceptible plants was 43:72:54 for the BC^2 and 20:11:12 for the RBC^2 progenies. Five additional BC^2 families with a total of 52 individuals included only susceptible plants. Genes for rust resistance were thus transmitted through the pollen as frequently as through the eggs of hybrid plants.

The ratios between resistant, intermediate, and susceptible plants in the BC^1, BC^2, and RBC^2 generations, together with the appearance of numerous completely susceptible plants, suggested that the inheritance of the resistance of *timopheevi* to stem rust might be simple, perhaps depending on not more than two major genes. To test this hypothesis, backcrossed progeny and some selfed progeny from single plants of known rust reaction were grown in 1944.

Only susceptible individuals were found in 438 progeny of susceptible plants of the BC^3, BC^2F_2, and BC^2F_4 generations. Apparently none of the resistance of *timopheevi* to races of rust occurring at Madison in 1944 was governed by recessive genes.

In 1944 almost no plants of the intermediate reaction were found, possibly because race 15B was not prevalent. The races of stem rust occurring in 1944 would not differentiate between the progeny of the intermediate and resistant plants of 1943. For example, 107 BC^3 progeny of 10 different resistant BC^2 plants segregated into resistant, intermediate, and susceptible individuals in the proportion of 56:2:49, respectively. The proportion for BC^3 progeny of 6 different BC^2 plants of intermediate resistance was 34:3:34. Both backcross ratios suggest that a single major gene governs resistance, if the somewhat higher incidence of rust on intermediate plants resulted from local environmental effects rather than genetic differences.

A large number of F_2 or F_3 segregates from BC^2 or RBC^2 and F_4 segregates from BC^1 plants were tested in 1944. The first would be expected to give F_2 ratios and in the F_3 and F_4 populations both segregating and homozygous lines could occur. In segregating lines the proportion of resistant : intermediate : susceptible plants was 1,952: 225:1,203. Although all attempts at a factorial analysis were unsuccessful, the proportions illustrate the ease with which stem-rust resistance was maintained.

Of the 11 BC^1F_4 families grown, 4 families of 11, 27, 45, and 89 individuals included only resistant plants, indicating that their BC^1F_3 parents were homozygous for resistance to stem rust. Several of these lines have been grown subsequently into the F_5 to F_7 generations and have been uniform for a high type of resistance, establishing the fact that homozygosity for resistance to stem rust occurred in F_3 individuals.

Although the data did not establish the number of genes governing the inheritance of *timopheevi* resistance to stem rust, they do suggest that relatively few major genes, possibly not more than 2, control the resistance to races which occurred at Madison in 1943 and 1944. At any rate a high order of resistance could be fixed by the F_3 generation

(from the first backcross), and a large proportion of resistant plants occurred in random populations after 4 backcrosses to *vulgare*. To determine the genic basis of resistance it is probable that studies of cytologically stable, 42-chromosome, homozygous resistant lines, crossed with other common wheats and tested with single races of rust will be necessary.

BUNT STUDIES

Studies of transmission of resistance to bunt were made in the greenhouse in 1943–44 and in 1944–45. A composite collection of bunt from three localities in Wisconsin was used as inoculum. All were smooth-spored types of the species *Tilletia foetida* (Wallr.) Liro. Seeds were dusted with chlamydospores of the causal organism and planted in soil maintained at approximately 10° C. The seeds were then covered with sand mixed with chlamydospores.

In the 1943–44 tests four groups of kernels were inoculated: F_1, BC^2, BC^1F_2 (o. p.), and RBC^2F_2 (o. p.) kernels. Progress and Reward, highly susceptible varieties, and the *vulgare* parent were grown as checks. More than 90 percent of the plants of these varieties were infected and showed the typical symptoms of the bunt disease. The F_1 plants were susceptible, but the symptoms were less pronounced than in the susceptible varieties. The plants were only slightly stunted if at all and very small spore balls were formed. Usually the disease did not involve the entire spike, but only a few spikelets.

BC^2 families varied widely in the proportion of bunt-infected plants; on the average they had slightly less disease than the *vulgare* parent. In 3 of 19 families tested, more than 50 percent of the individuals remained healthy, ratios of healthy to diseased plants in those families being 11:9, 4:1, and 10:9. The BC^1F_2 (o. p.) plants were considerably more resistant on the average than was the *vulgare* parent. In 10 of 15 families more than one-half of the plants remained healthy. In one family 20 of 23 and in another, 17 of 26 plants remained healthy. There was no indication that any of 18 RBC^2F_2 (o. p.) families possessed any more resistance to the disease than the common wheat parent, although their BC^1 parents apparently transmitted resistance to certain BC^2 and BC^1F_2 families. This suggests that resistance to bunt is less frequently transmitted by pollen than by eggs of segregating plants.

In 1944–45 tests all kernels obtained from bunt-free plants in 1943–44 were grown. Again, a few families had more healthy than bunted plants. Likewise, numerous partly bunted plants, which seemed to possess some measure of resistance to the disease, were found.

The foregoing results suggest that it may be possible to transfer some of the bunt resistance of *timopheevi* to *vulgare* types.

REGRESSION TOWARD THE RECURRENT PARENT IN CYTOLOGY AND FERTILITY

All chromosome numbers from 36 to 42 were observed in BC^1 plants (table 3). Because the male gametes from *vulgare* presumably had 21 chromosomes, functional female gametes of F_1 plants apparently possessed 15 to 21 chromosomes. Three additional backcrosses served to shift the mean chromosome number from 39.1 in the BC^1 generation to 41.6 chromosomes in the BC^4 population (table 3).

TABLE 3.—*Distribution of wheat plants of several generations according to meiotic behavior, frequency of micronuclei, proportion of good pollen, and fertility*

[The mean represents the mean value for each generation, while the range represents the maximum and minimum values for single plants within each generation]

Cytological character and seed set	Generation										
	Vulgare	BC 1	BC 2	BC 3	BC 4	RBC 2	RBC 3	BC 1 F 2	BC 2 F 2	F 2 (o. p.)	F 3 (o. p.)
Chromosome number:											
Mean	42.0	39.1	40.6	41.2	41.6	42.1	42.2	41.0	41.8	39.7	41.2
Range	None	36–42	38–44	37–44	41–43	41–43	42–43	38–44	40–43	37–42	40–43
Number of plants	11	78	88	68	19	31	13	22	15	18	12
Closed bivalents:											
Mean	19.3	10.1	13.5	17.1	18.2	16.0	17.9	14.1	16.7	10.2	14.5
Range	18.3–20.1	5.4–14.9	9.0–19.1	12.7–20.6	16.1–20.4	12.7–19.3	16.6–19.9	8.2–18.6	12.1–20.0	8.1–13.3	11.9–16.8
Number of plants	11	75	88	68	19	31	13	22	15	17	11
Open bivalents:											
Mean	1.5	4.4	3.9	2.4	1.8	3.5	2.4	4.3	3.2	4.3	3.5
Range	0.8–2.4	2.6–7.0	0.7–6.9	0.4–4.9	0.6–3.6	1.2–5.6	1.1–3.5	2.2–6.7	1.0–7.0	3.2–6.1	1.1–3.6
Number of plants	11	75	88	68	19	31	13	22	15	17	11
Total bivalents (open and closed):											
Mean	20.9	14.4	17.4	19.5	20.0	19.4	20.3	18.4	19.9	14.5	18.0
Range	20.4–21.0	10.6–18.3	13.9–20.8	17.1–21.0	18.8–21.0	15.2–20.8	19.2–21.0	14.9–20.8	16.8–21.0	12.9–16.8	16.9–20.7
Number of plants	11	75	88	68	19	31	13	22	15	17	11
Univalents:											
Mean	0.2	7.6	4.4	1.7	0.7	2.3	1.1	3.4	1.6	7.8	3.1
Range	0.1–0.2	3.5–13.9	0.2–9.7	0–5.9	0–2.4	0.2–5.5	0–2.9	0.4–5.3	0–5.8	4.3–13.3	1.3–6.1
Number of plants	11	75	88	68	19	31	13	22	15	17	11
Trivalents:											
Mean	0.01	0.7	0.4	0.1	0.2	0.2	0.1	0.3	0.1	0.9	0.7
Range	0–0.8	0–2.1	0–1.6	0–1.5	0–0.9	0.1–1.2	0–0.7	0–0.7	0–0.4	0–1.9	0–1.9
Number of plants	11	75	88	68	19	31	13	22	15	17	11
Micronuclei per quartet:											
Mean	0.2	2.3	1.6	0.9	0.3	0.7	0.7	1.4	1.2	----------	----------
Range	0.04–0.3	0.7–5.6	0.1–6.5	0.1–2.6	0.03–0.6	0.1–2.7	0.1–1.6	0.3–4.6	0.2–4.8	----------	----------
Number of plants	10	48	72	37	17	23	10	12	8	----------	----------
Percent quartets with no micronuclei:											
Mean	92	14	33	51	80	58	59	45	50	----------	----------
Range	91–98	0–75	0–90	5–95	56–97	14–91	21–91	3–95	0–88	----------	----------
Number of plants	10	48	72	37	17	23	10	12	8	----------	----------
Percent good pollen:											
Mean	95	23	51	65	----------	75	----------	69	----------	----------	----------
Range	51–97	1–90	4–95	2–96	----------	43–94	----------	14–94	----------	----------	----------
Number of plants	58	97	94	37	----------	10	----------	13	----------	----------	----------
Open-pollinated seed set (percent):											
Mean	94	9	38	56	----------	77	----------	43	----------	----------	----------
Range	73–96	0–85	0–93	15–95	----------	5–95	----------	5–91	----------	----------	----------
Number of plants	73	66	197	47	----------	28	----------	22	----------	----------	----------

| | | |

The range of chromosome numbers in the RBC^2 and RBC^3 generations was much smaller than for the BC^2 and BC^3 generations. Only 3 chromosome numbers were observed, 41, 42, and 43 (table 3, fig. 2, *H*). The average number of chromosomes for the RBC^2 and RBC^3 generations was slightly greater than 42. Thus, backcrosses in which the BC^1 plants were pollen parents resulted in a much more rapid return to the chromosome number of *vulgare* than did backcrosses with *vulgare* as the pollen parent.

In the BC^1F_2 and BC^2F_2 generations, the chromosome numbers clustered closer to 42 than in the comparable BC^2 and BC^3 generations. The average number of chromosomes in these generations was possibly slightly higher than in their backcrossed counterparts. This implies that a system of self-pollination in BC^1 plants might result in a slightly more rapid return to the chromosome number of *vulgare* than backcrosses, at least in the early generations.

Regression in meiotic behavior toward the recurrent parent was much more rapid in the RBC^2 and RBC^3 generations than in the comparable BC^2 and BC^3 generations. Even after three backcrosses, however, two with hybrid plants as the pollen parents, only about one-half of the population fell within the range of pairing of *vulgare*.

The meiotic behavior of BC^1F_2 and BC^2F_2 plants was as regular if not more regular than that of the comparable BC^2 and BC^3 generations (table 3).

Both backcrossed and selfed populations from backcross plants appeared to follow Kihara's scheme (p. 34). Variability in pairing was such that the most usual number of paired and unpaired chromosomes could not be easily established. However, when the mean number of paired and unpaired chromosomes (based upon analyses of 10 P. M. C.) was determined for each plant, it was observed that the sum of the bivalents and univalents was always equal to or exceeded 21. The average number of bivalents plus univalents in all backcrossed and selfed generations also was equal to or exceeded 21 (table 3). Thus, although monosomic plants were viable, there apparently was strong selectivity against nullisomic plants.

A further measure of cytological stability was obtained from the number of micronuclei per quartet of microspores and the number of quartets which had no micronuclei (table 3). BC^1 plants varied from an average of less than one micronucleus per quartet to an average of nearly six micronuclei per quartet. There was a steady decrease in the frequency of micronuclei with further backcrosses. Again, the return toward the recurrent parent was more rapid in the RBC^2 and RBC^3 generations than in the BC^2 and BC^3 generations. The limited data show that BC^1F_2 and BC^2F_2 plants had approximately the same number of micronuclei on the average as the BC^2 and BC^3 plants.

Large differences were found between plants of the several backcrossed generations in the amount of good pollen (table 3). Most BC^1 plants had a preponderance of aborted or incompletely developed pollen grains, and the anthers failed to open at maturity. A few BC^1 plants, however, fell within the range of variation of *vulgare* in percentage of good pollen. The amount of good pollen increased with further backcrosses until a majority of the BC^3 plants fell within the range observed for *vulgare*. Plants of the RBC^2 generation had an average of 75 percent of good pollen, exceeding the BC^2 and even the BC^3 generation. No data on pollen were obtained for the RBC^3

generation, but all plants had well-filled anthers that apparently dehisced normally.

The fertility of BC^1 plants was in general low (table 3). When 2 or more spikes of 58 different BC^1 plants were selfed, 30 of the plants failed to set any kernels and only rarely were more than 3 selfed kernels obtained from any one plant. In all, 157 kernels were obtained from 2,828 florets (6 percent seed set). When allowed to open-pollinate, the seed set was somewhat higher (9 percent), and only 10 plants of 66 set no kernels. Only 2 plants of 66 failed to set any kernels when backcrossed; hand-pollination of 2,288 florets yielded 648 kernels (28 percent seed set).

When pollen from 15 different BC^1 plants, selected for relatively large amounts of pollen, was used to pollinate florets of *vulgare*, no kernels were obtained with pollen from 3 plants, but the other 12 gave from 1 to 14 kernels per spike of 20 to 28 florets.

In general, BC^2 plants were more fertile than BC^1 plants (table 3). Of the 79 plants which were bagged to test self-fertility, 25 failed to set any kernels. In all, 530 kernels were obtained from 2,221 florets (24 percent seed set). Only 6 of 199 BC^2 plants, or 3 percent, failed to set any kernels when allowed to open-pollinate. The average fertility of these 199 open-pollinated plants was 38 percent (table 3). Seed set from backcrossing was 40 percent, or approximately the same as from open-pollination. Seventy-six BC^2 plants were backcrossed and none failed to produce kernels, although some were relatively low in fertility. The fertility of open-pollinated RBC^2 plants was rather uniformly high, 77 percent on the average. The range in fertility of BC^1 F_2 plants appeared to be the same as for the BC^2 generation (table 3).

Data for individual plants revealed that not until the BC^3 generation were individuals obtained which had 42 chromosomes that appeared to fall within the range of variation of *vulgare* in meiotic behavior, number of micronuclei, percentage of good pollen, and seed set. Because the data from individual plants were limited, the measurement of these factors was not sufficiently precise to determine with certainty that an individual had achieved the stability of *vulgare*. However, 4 BC^3 plants out of 68, 3 BC^4 plants of 19, and 5 RBC^3 plants of 13 appeared to meet these requirements. It is possible that more extensive data would have revealed cytological differences between these plants and *vulgare*.

A simple method of describing the mean cytological regression toward *vulgare* through four backcrosses is provided by the data presented in table 4. It is apparent that each backcross shifted the population approximately one-half the distance toward the recurrent parent when *vulgare* was the pollen parent. This relationship applied rather closely for several characters including chromosome number, closed bivalents, bivalents, univalents, mean number of micronuclei, percentage of quartets with no micronuclei, and percentage of good pollen. It is of interest to note that the genetic trend of a hybrid toward the recurrent parent upon continuous backcrossing in plants in which all chromosomes pair is also one-half per generation. The cytological regression toward *vulgare* was somewhat more rapid when backcross plants were the pollen parents in further backcrosses and is not well described by the above relationship.

TABLE 4.—*Cytological trend toward the recurrent parent compared with trend expected under the hypothesis that the difference between hybrid and recurrent parent is reduced one-half per generation of backcrossing*

Cytological character	Unit	F_1	BC^1	BC^2	BC^3	BC^4	*Vulgare*
Chromosomes	Number expected	35	38.5	40.3	41.1	41.5	42
	Number observed	35	39.1	40.6	41.2	41.6	42
Closed bivalents	Number expected	.....	11.3	15.3	17.3	18.3	.....
	Number observed	3.2	10.1	13.5	17.1	18.2	19.3
Bivalents	Number expected	.....	14.6	17.7	19.3	20.1	.....
	Number observed	8.3	14.6	17.4	19.5	20.0	20.9
Univalents	Number expected	.....	7.5	3.8	2.0	1.0	.....
	Number observed	14.8	7.6	4.4	1.7	.7	.1
Micronuclei per quartet	Number expected	.....	2.6	1.1	.6	.4	.....
	Number observed	5.0	2.8	1.6	.9	.3	.2
Quartets with no micronuclei	Percent expected	.....	47	70	81	87	.....
	Percent observed	1	15	33	51	80	92
Good pollen	Percent expected	.....	43	64	74	79	.....
	Percent observed	2	23	51	65	.....	84

CRITERIA FOR SELECTION

In early backcross generations there was little relationship between the cytological behavior of a particular plant and the cytological behavior of its offspring. For example, one BC^1 plant which had 42 chromosomes most frequently arranged as 12 IIc, 5 IIo, 5 I, and 1 III produced backcrossed offspring which varied from 40 chromosomes (9 IIc, 5 IIo, 9 I, and 1 III) to 43 chromosomes (17 IIc, 3 IIo, and 1 III). Not until an individual had 19 or more bivalent chromosomes was a similar or greater number of bivalents observed consistently in its offspring. This indicates that there is little to be gained toward increasing meiotic regularity by selecting for high pairing until at least 19 pairs have been achieved. It was not until the third or fourth backcross generation (table 3) that most plants reached this degree of meiotic stability.

Low frequency of univalents and of micronuclei similarly were not good criteria for selection in the early generations.

Fertility is relatively easily determined and if selection of the more fertile early-generation plants were effective in raising the general level of fertility, it would be a desirable criterion for selection. This was tested by arranging parental plants according to fertility percentages and tabulating the mean fertility and range in fertility of the offspring of each fertility class (table 5). The limited data indicate

TABLE 5.—*Relationship between the seed set of parental plants and the seed set of their backcrossed offspring*

BC^1 plants		BC^2 plants		
No.	Seed set in class centers [1]	No.	Seed set	
			Average [2]	Range
	Percent		*Percent*	*Percent*
6	5	8	11.6	1–28
11	15	59	45.4	0–85
6	25	31	40.5	2–86
1	35	8	15.6	2–48
2	45	22	30.3	4–66
1	55	36	51.7	5–91

[1] Seed set when backcrossed to *vulgare*.
[2] Open-pollinated seed set.

that progeny of the more fertile plants varied as widely in fertility as the progeny of less fertile plants; also there was no consistent trend in mean fertility. Observation of numerous families for which no numerical data are available corroborate the data of table 5. This suggests that in the early generations selection of only the more fertile plants is not particularly effective in increasing the fertility of the offspring.

THE BACKCROSS (VT)T

When 312 florets of F_1 plants were pollinated with pollen of *timopheevi*, 11 kernels were obtained (3.5 percent seed set). Seven of the kernels germinated to give plants that either resembled *timopheevi* closely, or were intermediate between *timopheevi* and the F_1 in morphological appearance.

A cytological examination was made of 3 of these plants. Two *timopheevi*-like individuals each had 29 chromosomes. The most usual pairing arrangement in both plants was 8 IIc, 3 IIo, and 7 I. An occasional trivalent was also observed. The third plant was intermediate between the F_1 and *timopheevi* in appearance and had 31 chromosomes, usually associated as 8 IIc, 9 IIo, and 7 I, plus a small fragment. The fragment associated with no other chromosome.

The 7 (VT)T plants were of varying fertility, and a few kernels were obtained from each when allowed to open-pollinate. Nineteen open-pollinated progeny were obtained, all of which resembled *timopheevi* very closely in morphological appearance. A cytological examination was made of 3 of these plants, each of which had 28 chromosomes usually conjugated as 11 IIc and 3 IIo, but occasional univalents were seen. All plants were highly fertile when allowed to open-pollinate. Apparently the return toward *timopheevi*, both morphologically and cytologically, was very rapid following one backcross to that species.

THE F^2 AND F^3 GENERATIONS

The 5 selfed kernels from F_1 plants (p. 37) were sown in the field in 1943. All germinated and grew into mature plants. One of these plants, designated F^2–1, resembled the parental common wheat variety very closely, including its susceptibility to stem rust. It had 42 chromosomes which paired fairly regularly (table 6), and 83 percent of the florets set seed. The following season 18 progeny of this plant were grown. They also closely resembled the parental common wheat variety and all were highly fertile.

The 4 other F_2 plants, designated F_2–2, F_2–3, F_2–4, and F_2–5, differed greatly from one another. All 4 plants had the general gross morphological appearance of *timopheevi*, but they possessed thickened plants parts which often characterize autopolyploids. Plants F_2–2, F_2–4, and F_2–5 had 45, 46, and 49 chromosomes respectively. Conjugation was irregular, univalents and multivalents occurring frequently (table 6). Plant F_2–3 was completely sterile. The other 3 plants set a few kernels when allowed to open-pollinate but they set no kernels under bags. All of the kernels obtained from the 3 partly fertile plants were sown in 1944 and 22 F_3 plants were obtained. These F_3 plants varied amongst themselves even more widely than had their parents, but again all the plants had the gross

general morphological appearance of *timopheevi* (fig. 6, *B* and *C*). Some had thickened plant parts (fig. 6, *B*), whereas others were quite slender and stunted (fig. 6, *C*). All were highly sterile. Seven of these F_3 plants that were examined cytologically were found to have the high chromosome numbers and irregular meiotic behavior that characterized the F_2 (table 6).

TABLE 6.—*Distribution of chromosomes according to type of association in plants of the F_2 and F_3 generations from Triticum vulgare × Triticum timopheevi*

Plant	Average and range	2*n* chromosomes	Type of chromosome association							
			IIc	IIo	II	I	III	IV	V	VI
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
F^2 GENERATION (from selfed F_1 plants)										
F_2-1	Average	42	16.6	3.2	19.8	2.0	0	0.1	0	0
	Range		12–21	0–6	18–21	0–6	0	0–1	0	0
F_2-2	Average	45	12.9	3.7	16.6	6.4	1.0	0.6	0	0
	Range		11–16	0–7	13–20	4–11	0–2	0–2	0	0
F_2-4	Average	46	11.1	4.3	15.6	7.9	1.3	0.7	0	0.1
	Range		9–15	2–6	12–18	2–12	0–2	0–2	0	0–1
F^2-5	Average	49	9.6	5.2	14.8	10.1	2.2	0.4	0.1	0.1
	Range		6–14	3–9	9–20	6–15	0–4	0–1	0–1	0–1
F_3 GENERATION (from open-pollinated F_2 plants)										
F_2-2-3	Average	45	9.5	2.6	12.0	2.9	2.0	0.5	0	0
	Range		6–14	3–9	10–18	4–9	0–5	0–1	0	0
F_2-2-8	Average	45	15.0	3.5	18.5	3.5	0.5	0.8	0	0
	Range		11–19	2–6	15–21	2–4	0–1	0–2	0	0
F_2-4-4	Average	43	12.3	5.5	17.8	2.5	1.0	0	0	0
	Range		10–16	3–9	16–19	2–4	1–1	0	0	0
F_2-4-5	Average	45	9.5	6.8	16.3	8.2	1.0	0	0.3	0
	Range		6–14	4–9	12–19	6–10	0–2	0	0–1	0
F_2-4-7	Average	46	14.5	4.5	19.0	3.0	0.6	0.4	0.2	0.1
	Range		12–17	3–7	17–21	0–5	0–1	0–1	0–1	0–1
F_2-5-1	Average	41	15.3	4.1	19.4	1.2	0	0	0	0
	Range		12–18	2–8	18–20	1–5	0	0	0	0
F_2-5-2	Average	50	11.3	5.8	17.1	4.2	1.5	1.2	0	0
	Range		7–14	4–9	14–20	2–7	0–2	0–2	0	0

The origin of the F_2 kernels may be inferred from the type of gametes produced by the F_1 plants in reciprocal backcrosses to *vulgare*. It has been shown that the functional pollen of the F_1 had 14 chromosomes and that the functional eggs had 15 to 21 chromosomes. Thus self-fertilized kernels from the F_1 would be expected to receive 14 chromosomes from the pollen and 15 to 21 chromosomes from the eggs, making a total of 29 to 35 chromosomes. Morphologically they would be expected to resemble *timopheevi* or to be intermediate between *timopheevi* and the F_1. The plants obtained deviated widely from the types expected from self-fertilization in F_1 plants, both morphologically and cytologically. Consequently some explanation other than self-fertilization by reduced gametes must be sought to account for these kernels.

The single *vulgare*-like plant had 0 to 6 univalent chromosomes (mean of 2) and occasionally formed the maximum of 21 closed pairs. It could have resulted from the fertilization of an egg which received nearly exactly the 21 chromosomes of *vulgare* by a pollen grain of similar constitution. However, it was not possible to demonstrate by fairly extensive backcrossing that functional 21 chromosome pollen grains were formed by the F_1. Because the anthers of the F_1

FIGURE 6.—Spikes: *A, timopheevi; B,* F_2–4–7, a 46-chromosome plant; *C,* F_2–2–3, a 45-chromosome plant; *D, vulgare.*

were leathery and tough and did not dehisce, escape of the occasional "good" pollen grains seems unlikely. The restitution (doubling) of a reduced female gamete with 21 *vulgare* chromosomes (or nearly so) and subsequent parthenogenetic development is an alternative hypothesis that is more plausible.

The other 3 F_2 plants had about twice as many bivalent chromosomes and much more frequent multiple associations than did the F_1 plants. The restitution of female gametes after a reduction division involving the loss of several chromosomes, mostly chromosomes of *vulgare*, is a possible explanation which is in accord with the pairing observed (table 6). Another possible explanation involves the union of an unreduced or partly reduced female gamete with a 14-chromosome male gamete of the F_1. The union of a 14-chromosome male gamete with an unreduced egg (35 chromosomes), or with partly reduced eggs that contained 32 or 31 chromosomes, would give plants with 45, 46, and 49 chromosomes, the numbers observed in plants F_2–2, F_2–4, and F_2–5. These plants would have the diploid *timopheevi* complement plus a monoploid *vulgare* complement (minus 3 and 4 *timopheevi* or *vulgare* chromosomes in plants F_2–2 and F_2–4). The pairs would probably be 14 bivalents from *timopheevi* chromosomes depending on the chromosomes that were "minus," plus occasional bivalents from autosyndetic conjugation of *vulgare* chromosomes and multivalents from combined auto- and allosyndesis.

The gross resemblance of the open-pollinated F_3 plants to *timopheevi* suggested that they resulted from the fertilization of partly reduced eggs with high chromosome numbers by pollen grains from nearby plants of *timopheevi*. They could possibly, however, have come from restitutions and parthenogenetic development of partly reduced eggs, or from self-fertilization.

THE F_2 (O. P.) GENERATION OR FIRST NATURAL BACKCROSS GENERATION

In the summer of 1942, a row of F_1 plants was grown between rows of the 2 parents. From 329 open-pollinated spikes of the F_1 plants 75 kernels were obtained. Because the F_1 was late in maturity, its flowering period coincided more closely with that of *timopheevi* than with that of the earlier maturing *vulgare* parent. It was not unexpected, then, that 25 of the 30 plants obtained from these kernels, which were almost certainly from outcrosses, resembled *timopheevi*. The variation in appearance of these 25 natural backcrossed plants was the same as that of plants from controlled backcrosses to *timopheevi*. Of the remaining 14 F_2 (o. p.) plants, 8 were intermediate between the F_1 and *timopheevi* but more closely resembled the F_1, and 6 resembled *vulgare*. The former probably were from eggs which had largely a *vulgare* complement of chromosomes fertilized by male gametes from *timopheevi*. The latter were probably from natural backcrosses to *vulgare*.

During the 1943 season the following variations were made in order to obtain a larger proportion of *vulgare*-like F_2 (o. p.) plants. About 100 F_1 plants were interplanted with the variety Marquis. By delaying the planting date of Marquis and cutting it back, heading was made to coincide with that of the F_1 plants; 174 F_2 (o. p.) kernels were found on the F_1 plants. A random group of 84 was sown in 1944 and 31 plants were obtained. Twenty-five individuals with awnlets like those of Marquis were observed, providing evidence that natural outcrosses to Marquis gave rise to the majority of the kernels (fig. 7, B). Four plants which resembled the common wheat parent were found (1 shown in fig. 7, *D*), 1 *timopheevi*-like plant (fig. 7, *C*), and 1 plant

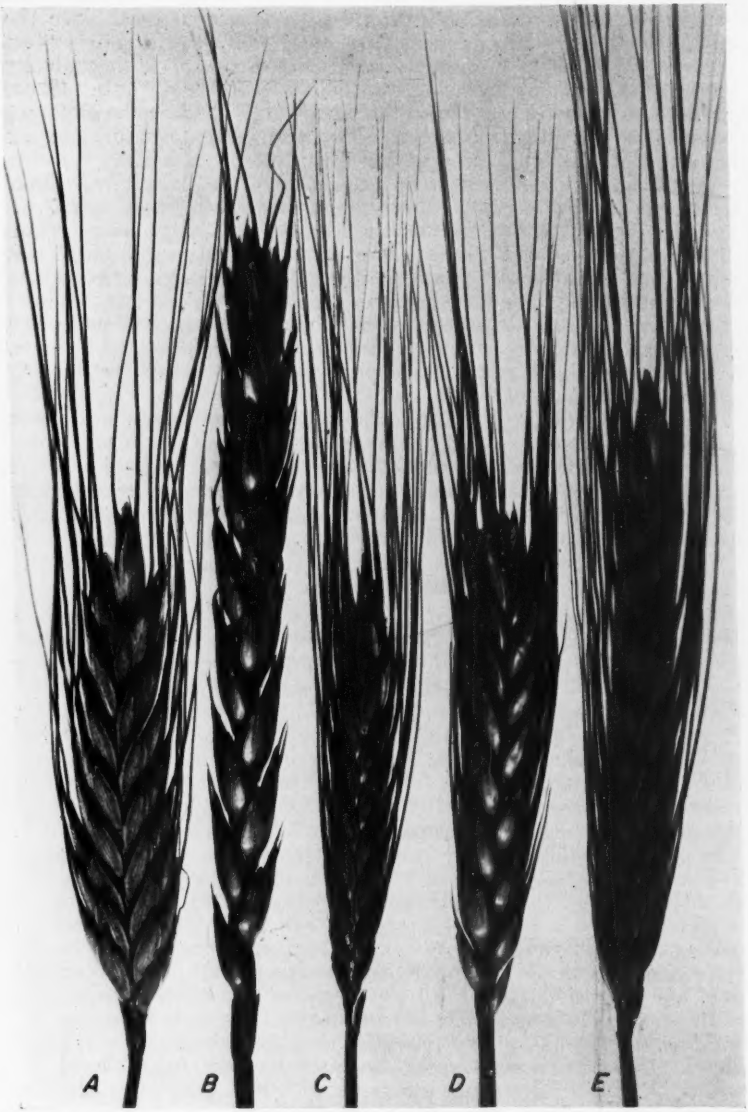

FIGURE 7.—Spikes: *A*, 28-chromosome *timopheevi*-like F_3 (o. p.) plant; *B*, awnleted F_2 (o. p.) plant with 38 chromosomes, probably from an outcross to Marquis; *C*, F_2 (o. p.) plant with 30 chromosomes, probably from an outcross to *timopheevi*; *D*, F_2 (o. p.) plant with 40 chromosomes, probably from an outcross to the common wheat parent; *E*, F_1-like F_3 (o. p.) plant with $2n=21$ chromosomes, probably parthenogenetic in origin.

which was intermediate between the F_1 and *timopheevi*. Since the common wheat parent and *timopheevi* were growing within a few feet of some of the F_1 plants, occasional backcrossing to them probably explains the few plants which did not resemble Marquis. These results were very different from the results of 1942 when the F_2 (o. p.) population was largely *timopheevi*-like, and serve to emphasize the control of natural backcrossing that is possible.

A cytological examination was made of 20 of the 31 F_2 (o. p.) plants which were grown in 1944. The single *timopheevi*-like plant (fig. 7, *C*) and the plant intermediate between the F_1 and *timopheevi* each had 30 chromosomes. The usual pairing for both plants was 8 IIc, 5 IIo, and 4 I. Chromosomes numbers of the 18 *vulgare*-like plants which were examined cytologically are given in table 3. The distribution of chromosome numbers appeared to be about the same as for the BC^1 generation. Similarly the variation in numbers of closed bivalents, total bivalents, and univalents approximated the values for the BC^1 generation (table 3).

The fertility of the F_2 (o. p.) plants varied considerably and was correlated with morphological appearance. Plants which resembled the F_1 were completely sterile. Nearly half of the *vulgare*-like and *timopheevi*-like plants, however, set some kernels. The range in fertility of *vulgare*-like F_2 (o. p.) plants was approximately the same as that of the comparable BC^1 generation.

THE F_3 (O. P.) GENERATION

The appearance of F_3 (o. p.) plants was related to the appearance of their F_2 (o. p.) parents. *Timopheevi*-like F_2 (o. p.) plants had F_3 (o. p.) offspring which usually resembled *timopheevi* but occasionally were like the F_1 in appearance. Eight such F_3 (o. p.) plants were examined cytologically. Five *timopheevi*-like plants (1 shown in fig. 7, *A*) had 28 chromosomes each and 1 plant had 29. The usual pairing was 14 bivalents in the 28-chromosome plants and 14 bivalents plus 1 univalent in the 29-chromosome plant, although some univalents were observed in all 5 plants. One F_1-like plant had 35 chromosomes which usually associated as 7 IIc, 6 IIo, 6 I, and 1 III. This plant probably resulted from an outcross with *vulgare*. Another F_1-like plant (fig. 7, *E*) had only 21 chromosomes, usually associated as 2 IIc, 3 IIo, 8 I, and 1 III, and probably was of parthenogenetic origin.

Progeny of *vulgare*-like F_2 (o. p.) plants usually resembled *vulgare* more closely than did their parents, but occasional plants resembled the F_1. *Vulgare*-like F_3 (o. p.) plants probably resulted from selfing of F_2 (o. p) plants or from outcrosses to the common wheat parent or Marquis. F_1-like plants probably were from outcrosses to *timopheevi*. The chromosome numbers and meiotic behavior of 11 *vulgare*-like F_3 (o. p) plants are given in table 3. The cytological regularity was about the same as that of the corresponding BC^2 generation.

In the F_2 (o. p.) and F_3 (o. p.) generations, all *timopheevi*-like or F_1-like plants were highly resistant to stem and leaf rust. Segregation for stem rust resistance occurred, however, among the *vulgare*-like individuals of these generations. Of 6 *vulgare*-like F_2 (o. p.) plants grown in 1943, 3 were highly resistant and 3 were intermediate; in

1944 there were 5 resistant, 12 intermediate, and 12 susceptible plants. Three *vulgare*-like F_3 (o. p.) families grown in 1944, all from highly resistant F_2 (o. p.) plants, segregated as follows (resistant: intermediate: susceptible): 22:3:4, 50:0:11, and 4:0:3. Thus there was no more difficulty in maintaining stem rust resistance in a large proportion of the natural backcrossed than in the artificially backcrossed populations.

A few of the stem and leaf rust resistant F_3 (o. p.) plants were selected and open-pollinated progeny grown for two additional generations. Although there was no difficulty in fixing rust resistance in highly fertile lines, few agronomically desirable types were obtained. Hybrids were then made between the better lines and several common wheat varieties and the F_1's were backcrossed to the common wheat varieties one or more times. Segregates from these backcrosses resembled the recurrent parent agronomically. Far less effort in transferring stem and leaf rust resistance to agronomically desirable types was required by this system than by backcrossing in early generations when fertility was poor.

THE BC^2F_4 GENERATION

Cytological observations were made on one of the most promising BC^2F_4 lines. It bred true for resistance to both leaf and stem rusts and to mildew, was completely fertile, and of fair agronomic type. Six plants were examined, all of which had 21 pairs of chromosomes (fig. 2, *I*), and 96 percent or more of the quartets were free from micronuclei. This line appeared to be as stable cytologically as the *vulgare* parent. In its hybrids with Marquis and Reward, 21 pairs were observed uniformly, and 97 percent or more of the quartets were free from micronuclei in 3 F_1 plants of each hybrid. Thus the rust and mildew resistance of *timopheevi* can be incorporated into cytologically stable 21-chromosome wheats. Moreover, because these derivatives from *timopheevi* are cytologically regular in hybrids with certain *vulgare* wheats, there should be no cytological difficulties if they are used as disease-resistant parents.

DISCUSSION

The special position that *Triticum timopheevi* occupies among the 28-chromosome wheats, first recognized from cytological evidence, is further substantiated by the breeding behavior of this species in its hybrid with *T. vulgare*, agricultural variety 2666A2–2–15–6–3. This hybrid does not follow Kihara's (*18*) scheme established from researches upon other pentaploid wheat hybrids in which the parental chromosome numbers are regained following selfing of the hybrid. Rather the hybrid of *T. vulgare* $\times$ *T. timopheevi* is probably completely male-sterile in nature as a result of indehiscence of the anther, and the few kernels obtained apparently are produced by other than the usual sexual process. However, once the population had been shifted toward either parent by backcrossing with the F_1 as the female, it was found that the return to the 42-chromosome or 28 chromosome condition was according to Kihara's scheme.

This hybrid also differs significantly from other pentaploid wheat hybrids in the character of the populations obtained from backcrossing to the common wheat parent. In other pentaploid hybrids

all chromosome numbers from 14 to 21 are represented in functional male gametes of F_1 plants, although the most numerous functioning gametes have chromosome numbers approaching one or the other parent (*34*, *36*). It was demonstrated in the present study that the only male gametes that functioned possessed 14 chromosomes. As a consequence no progress toward the *vulgare* type was noted from backcrosses made with the F_1 as the pollen parent. When the *timopheevi-vulgare* F_1 was backcrossed with pollen from *vulgare*, the results diverged less from other pentaploid hybrids than in the reciprocal backcross, but fewer pairs of chromosomes were observed and the average fertility of the backcrossed population was lower. The relatively remote relationship between *timopheevi* and common wheat is thus expressed in their hybrid in low chromosome pairing and low fertility, expecially in the male gametes. Both factors serve as major barriers to the transfer of genes.

Nevertheless, transference of genes governing stem and leaf rust resistance by successive backcrosses was obtained. However, only a few genes govern rust resistance and these genes were transmitted with approximately equal frequency through both male and female gametes of early-generation backcross plants. Because functional male gametes of backcross plants were genetically and cytologically similar to *vulgare* gametes, backcrosses in that direction acted as a fine sieve which passed genes controlling rust resistance but screened out nearly all other *timopheevi* genes. Accordingly, it was possible to fix stem and leaf rust resistance rapidly in cytologically stable 42-chromosome types by backcrosses with early generation backcross plants as pollen parents.

It is possible that genes governing stem- and leaf-rust resistance are located in one or more of the few *timopheevi* chromosomes which appear to have nearly exact homologues in *vulgare*. Presumably such chromosomes could be substituted for their homologues without seriously decreasing the viability of either male or female gametes of backcrossed plants. Because mildew resistance was found in lines derived from this type of backcross, the genes concerned can also be presumed to be located in chromosomes of *timopheevi* which have homologues in *vulgare*.

Other characters of *timopheevi* studied, including bunt resistance, were less easily transferred to *vulgare* types. If the genes concerned were located in *timopheevi* chromosomes which pair sporadically with chromosomes of *vulgare*, the gametic unbalance and consequent gametic elimination which inclusion of partial homologues might cause may account for the failure of such characters to appear in backcrossed generations. Another possible explanation is that these characters were governed by numerous genes. Anderson (*2*) has discussed the reasons why transfers of characters from one species to another are likely to be difficult when a character is controlled by several genes.

The transfer of genes in chromosomes which completely lack homology depends upon the substitution or addition of a complete chromosome pair, as demonstrated by Gerstel (*6*, *7*). The place of "alien addition" and "alien substitution" races in practical plant breeding remains to be determined.

Although stem-rust resistance was transferred to apparently stable 42-chromosome *vulgare* types by successive backcrosses, utilization of

natural backcrosses proved more efficient in time and labor for this transfer. Because the F_1 hybrid was nearly completely male-sterile under field conditions, any kernels produced upon F_1 plants most likely were from outcrosses. By interplanting with the desired recurrent parent and with proper isolation, naturally backcrossed kernels were obtained more easily than by hand-pollinations. A mixture of self-pollinated and backcrossed kernels was obtained when these seeds were again interplanted with the recurrent parent. Among these plants were numerous rust-resistant individuals of moderate to high self-fertility. However, undesirable characters, such as lateness of maturity and weak straw, were usual and few or no agronomically desirable lines could be isolated by selection in 2 additional open-pollinated generations. Further improvement was accomplished by artificial backcrosses to common wheat varieties. Under the system outlined above, natural backcrossing was utilized in early generations to lessen the labor required, while artificial backcrosses were employed when fertility had been restored and hand-pollinations were more fruitful.

SUMMARY

In the hybrid between *Triticum vulgare* and *T. timopheevi* there were marked differences in chromosome numbers and pairing, fertility, and morphological appearance in generations derived by selfing and reciprocal backcrosses to *vulgare*. This hybrid differs significantly from other pentaploid hybrids in its breeding behavior.

The rare kernals produced upon selfed F_1 plants appeared to have arisen by an abnormal sexual process.

Backcrosses obtained by using pollen of the F_1 on *vulgare* were nearly indistinguishable from the F_1 in morphological appearance cytological behavior, and fertility.

Plants obtained by using pollen of *vulgare* on the F_1 were variable but in general intermediate between the F_1 and *vulgare*. The resemblance to *vulgare* increased through four similar backcrosses. The mean difference in cytological behavior and fertility between plants of each backcrossed generation and *vulgare* was decreased by half per generation of backcrossing to *vulgare* when backcrossed plants were the seed parents.

When the second backcross was made with the first backcross plants as the pollen parents, the resulting population resembled *vulgare* more closely than did the reciprocal backcross and was more regular cytologically and more fertile. Resistance to stem and leaf rusts and mildew was maintained in this type of backcross, but nearly all other characters of *timopheevi*, including resistance to bunt, were eliminated. This system of mating thus allowed a rapid transfer of *timopheevi* resistance to stem and leaf rusts and mildew to 42-chromosome *vulgare* types.

Little was gained toward greater fertility or meiotic stability by selecting the more fertile or more cytologically regular plants in early generations. Selection in early generations should be only for the specific genes sought.

The self-sterile F_1 may be satisfactorily backcrossed by planting in proximity to the desired male parent.

Despite the poor pairing and low fertility of the F_1, genes for resistance to stem rust, leaf rust, and mildew possessed by *timopheevi* were

transferred to *vulgare*. This was accomplished most efficiently by three or four generations of natural backcrossing resulting in fertile disease-resistant types which were then backcrossed successively to *vulgare* until the genes for resistance were stabilized in an otherwise *vulgare* genotype.

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RED STEM CANKER OF COWPEA, CAUSED BY PHYTOPHTHORA CACTORUM [1]

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INTRODUCTION

In 1944 a disease not heretofore observed was found causing injury and death to a considerable number of plants in a field of cowpeas (*Vigna sinensis* (Torner) Hassk.) at Experiment, Ga. The following year the disease appeared in another field but it was less destructive, occurring most commonly in low, poorly drained areas. The first year there was no apparent correlation between the prevalence of the disease and the soil moisture; then the disease was present on a hillside where soil and air drainage were excellent. The disease was not observed in 1946 or 1947, probably because few growing cowpea plants were seen and little time was spent searching for the disease. So far as known the disease is not generally destructive, but it is potentially capable of doing considerable damage. This paper reports the cause of the disease and other pertinent facts about it.

SYMPTOMATOLOGY

The disease under discussion is easily recognized by the long, reddish lesions that may occur on any part of the stem or the petioles of cowpeas. This characteristic red color suggested the common name, red stem canker. The canker may involve part or all of the circumference of the stem and may extend from the base to the tip of the plant affected (fig. 1). At first the infected tissue looks water-soaked, but it soon becomes "maroon" [3] or "diamine brown" (*6*).[4] The centers of the old lesions frequently are lighter than the margins; often they are nearly "brick red" or "prussian red," but the center of a single lesion may vary considerably in color. When the tissue dries out the lesions may split longitudinally. Frequently the center of a lesion is invaded by one or more different fungi, largely if not entirely saprophytic.

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[2] The writer is indebted to B. L. Wade, U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., for the seed of the cowpea varieties used in these investigations.

[3] Throughout this paper the quoted colors are from Ridgway (*6*).

[4] Italic numbers in parentheses refer to Literature Cited, p. 75.

FIGURE 1.—Cowpea plants naturally affected with the red stem canker: *A–C*, Plants that have become one-sided because of killing of one side by the red stem canker fungus; *D*, plant practically killed. × approximately ⅕.

Infected stems of young plants may be girdled and the plants killed (fig. 2, *B*). Often only one side of a plant is seriously injured and its branches are killed, and thus a one-sided plant is produced (fig. 1, *A–C*). Sometimes the apical bud is killed and elongation of the main stem stops. Usually the lesions appear first a short distance above the soil surface and the base of the stem is not injured. When infection does take place below the surface of the soil, the fungus grows up the stem and produces a narrow lesion which becomes broader when it is above the soil surface (fig. 3).

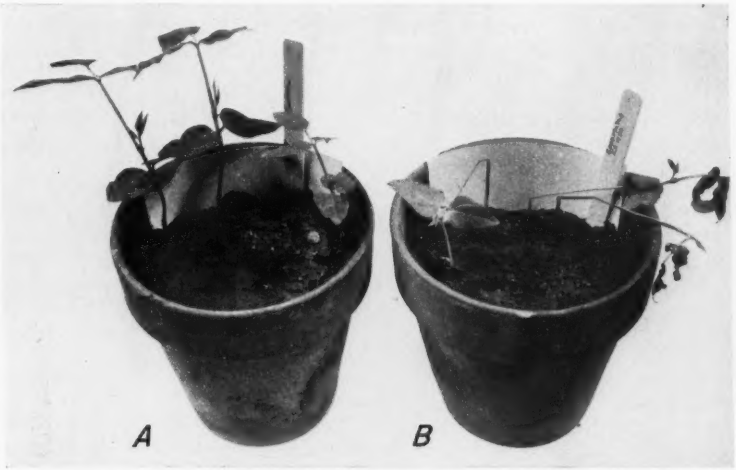

FIGURE 2.—*A*, Uninoculated cowpea seedlings; *B*, seedlings of same age inoculated with the red stem canker fungus and photographed 5 days after inoculation. × approximately ¼.

The symptoms of red stem canker differ from those of bacterial canker as described by Burkholder (*2*) and Dunlap [5] and described and illustrated by Hoffmaster (*4*) in that the lesions of the former are red and are not swollen or cracked. Red stem cankers differ from the lesions produced by *Rhizoctonia* in that the latter are largely underground. Typical cankers caused by *Macrophomina phaseoli* (Maub.) Ashby on cowpea differ from red stem cankers in that they have chocolate-brown borders and ashen centers and their surfaces often are thickly dotted with pycnidia.

ISOLATIONS AND INOCULATIONS

The surface of some lesions shows no change from that of the healthy stem except a uniformly reddish color; therefore, it gives no clue to the probable causal agent. On other lesions there occur lighter areas that are often covered with fungus fruiting bodies.

[5] DUNLAP, A. A. TWO BACTERIAL DISEASES IN TEXAS. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 27: 274. 1943. [Processed.]

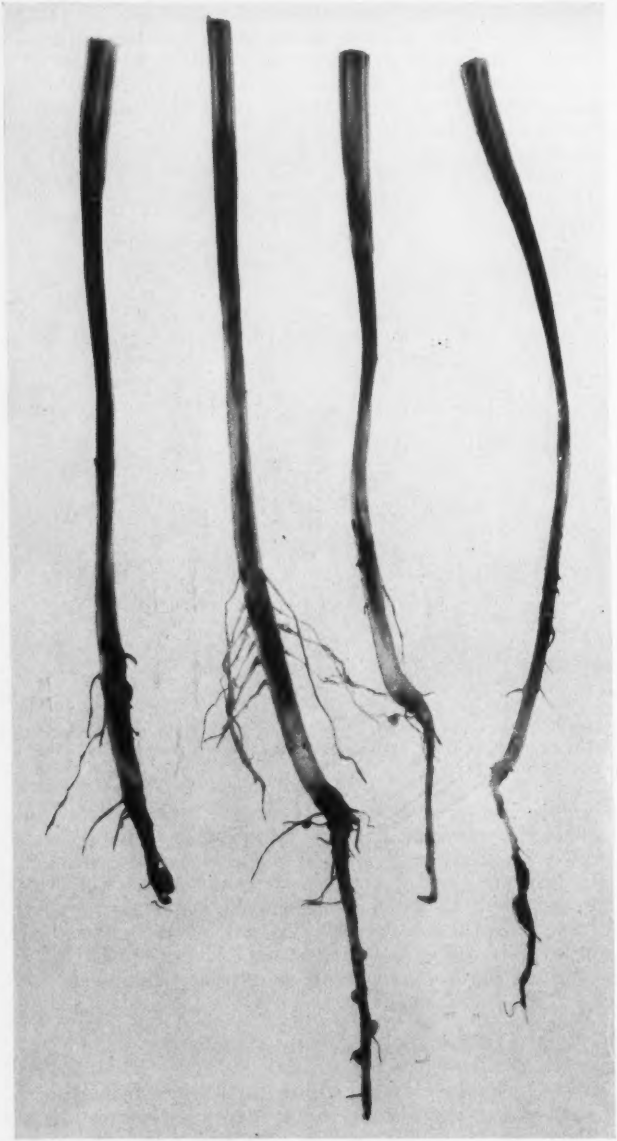

FIGURE 3.—Cowpea stems inoculated just below the surface of the soil with the red stem canker fungus. Note that the fungus produced lesions at the points of inoculation and then grew upward forming narrow, reddish lesions that became broader after reaching the above-ground parts of the stems. × 1⅒.

These may be the imperfect stage of an anthracnose fungus, sometimes a type with curved spores and at other times a type with straight spores resembling those of *Glomerella cingulata* (Ston.) Spauld. and Schrenk. The ascospore stage of the latter fungus is sometimes present. Under field conditions a coating of spores of *Fusarium* sp. also may be found on the older lesions. Less frequently fruiting bodies of other fungi such as *Alternaria*, *Phoma*, *Helminthosporium*, or *Macrophomina* are present. Of these fungi the curved-spored *Colletotrichum* and a species of *Fusarium* were found most consistently. These two fungi were isolated, but inoculation experiments showed that they were nonpathogenic. Further search showed that oospores of a phycomycetous fungus were consistently present in the diseased tissue; this fungus was then isolated and used in inoculation experiments.

On December 21, 1945, five cowpea plants of an unidentified variety growing in pots in the greenhouse were inoculated with this phycomycetous fungus by placing bits of agar on which it was growing against the unwounded stem near the surface of the soil; then the pots were held in a moist chamber for 70 hours. Control plants were treated in the same manner except that sterile agar was placed against the stems. The five inoculated plants were all dying 4 days after inoculation, the stems being nearly girdled at the point of inoculation. The fungus had penetrated the tissue in both directions from the point of infection. There was no injury to the control plants. The fungus used in the inoculations was reisolated from the diseased tissue.

The phycomycetous fungus used in the experiment just described and the reisolate were used in a second inoculation experiment begun on January 4, 1946. Three plants were inoculated with each fungus, and three others were held as controls. The fungus growing on agar was placed against the stem of each seedling a short distance above the soil surface. Sterile agar was placed against the stems of the control plants. All plants were held in a moist chamber for 68 hours. Four days after the inoculations were made five of the six inoculated plants had stem cankers typical of those seen under field conditions. Five days after inoculation the stems of the three plants inoculated with the original isolate were so badly decayed that their tops had fallen over (fig. 2, *B*). The fungus under investigation was reisolated from plants in both sets of inoculations. The tissue of most of the lesions contained oospores typical of the fungus used in making the inoculations. Thus the disease was reproduced by the fungus isolated, the fungus was again recovered, and this reisolate reproduced the disease and was recovered.

In a third inoculation experiment begun on March 11, 1946, 12 plants were inoculated with the original isolate and 10 of them became infected. At the same time 12 plants were inoculated with the reisolate from the second experiment and all of them became infected.

These experiments and a number of others in which plants were inoculated to test varietal susceptibility proved conclusively that the phycomycetous fungus is the cause of the disease. Infection usually was evident 3 or 4 days after inoculation. Seedling cowpea plants were most susceptible, but the youngest tissues of older plants, even those setting seed, were susceptible also. Some of the lesions a

week old were 2 to 3 inches or more in length and often involved the entire diameter of the stem so that it broke over. In other cases the lesions remained superficial. Figure 4 shows lesions on cowpea stems inoculated with a reisolate of the original culture. At first the lesions were reddish green. The amount of red varied with the lesion, some being "liver brown" and others "yellowish olive." Often affected plants that were not girdled and killed were more or less dwarfed or unsymmetrical. Some stems had lesions 5 to 8 inches long 18 days after inoculation.

In order to determine the relative resistance of different varieties of cowpea to the phycomycetous fungus being studied, several varieties were grown in pots in sterilized field soil in the greenhouse and inoculated as described previously when the seedlings were putting out their first trifoliate leaves. As shown in table 1, a high percentage of infection of most of the varieties tested was obtained. Alalong, Speckled Crowder, and Purple Hull appeared to be somewhat resistant in these small-scale tests. None of the 31 control plants of 8 varieties became infected.

TABLE 1.—*Results of inoculating various varieties of cowpea with the red stem canker fungus*

Variety	Plants inoculated	Plants infected	Variety	Plants inoculated	Plants infected
	Number	*Percent*		*Number*	*Percent*
Alabrowneye	7	71	Cream Crowder-Lady	8	100
Alabunch	15	93	Cream-longpod	10	100
Alalong	8	25	Early Dixie Queen	8	100
Blackeye Wilt-resistant	7	100	Jackson 21	4	100
Blue Goose	13	92	Purple Hull	3	33
Brabham	17	100	Purple Hull Bunch	14	100
California Blackeye No. 5	3	67	Sixweeks	15	93
Chinese Red	16	75	Speckled Crowder	15	27
Cream	1	100	Sugar Crowder	19	90
Cream Crowder	3	67	Whippoorwill	8	100

CULTURAL CHARACTERISTICS AND MORPHOLOGY OF THE FUNGUS

The causal fungus is easily isolated from the young red stem cankers by ordinary cultural methods, but this is no longer true after the tissue has been invaded by bacteria and secondary fungi. The fungus grows well on most media. The mycelium is white or slightly brownish on some media and varies in luxuriance with the medium. Oospores are formed much more abundantly on corn-meal agar than on oatmeal agar. Moderately good growth takes place on Leonian's malt agar, but oospores are formed sparsely on it.

Usually on the above-mentioned agars the hyphae range from 4μ to 4.5μ in diameter, but sometimes hyphae of irregular diameter 8μ or more across occur. There also are numerous short hyphal branches 5μ to 15μ long. Young branches may be greatly constricted where they join the mother hypha, then become broader than the latter a short distance from it, and taper gradually to a blunt point.

When a small piece of agar on which the fungus is growing is placed in water sporangia are formed on sporangiophores which are simple at

FIGURE 4.—*A*, Uninoculated stem of cowpea; *B* and *C*, cowpea stems inoculated with the red stem canker fungus in the greenhouse and photographed 8 days after inoculation. × 1¼.

first but later branched and vary in length from 50μ to 1,000μ. When a sporangium is to all appearances mature a secondary sporangiophore emerges from the first a short distance below the sporangium. This secondary sporangiophore elongates until it reaches a short distance above the primary sporangium, and then it forms a secondary sporangium. This process continues until four or more sporangia are produced, each a short distance above the other. The sporangia thus produced are lemon-shaped, oval, or elliptical and have a distinct papilla, usually terminal but sometimes lateral. Fifty sporangia measured 25μ to 50μ in diameter, but more commonly they ranged from 30μ to 40μ.

Germination in water is by formation of zoospores. These are completely formed within the sporangium and emerge from it in a mass but scatter almost immediately. Frequently several zoospores fail to escape through the apical pore with the others. Some of these escape later, but others never do. Usually the zoospores remain active for about half an hour and then round off; after a short rest period they germinate.

When grown on malt agar, the oogonia are pyriform or subspherical and measure 18μ to 30μ by 21μ to 31μ (50 measured; average, 25.3μ by 26.7μ). The antheridia are largely paragynous, but sometimes amphigynous, and are attached to the lower half of the oogonia. They remain distinct and appear to be empty when the oospores are formed.

The oospores are slightly yellowish to almost colorless. They are subspherical and have a smooth wall 1.0μ to 2.2μ thick. The diameter measurements were as follows:

Source of oospores:	*Oospores measured (number)*	*Range (μ)*	*Average (μ)*
Vigna sinensis	50	13.7 to 23.1 by 13.7 to 25.2	19.6 by 20.2
Oatmeal agar	55	14.7 to 27.3 by 15.8 to 29.4	21.9 by 22.3
Leonian's malt agar	65	14.7 to 27.3 by 14.7 to 27.3	20.3 by 21
Corn-meal agar	50	14.7 to 26.2 by 16.8 to 27.3	20.9 by 21.5

They averaged 20.8μ.

Few chlamydospores were seen.

The effect of temperature on growth of the fungus was determined by growing a single isolate in five petri dishes at each of various temperatures. On Leonian's malt agar the fungus failed to make any growth at 7° to 8° C. in 9 days; its optimum for vegetative growth was between 26° and 28°, although its growth at 31.5° was only a little slower. Oospores were formed more abundantly below 25° than above. The fungus grew well at 31.5°, but it made very slight growth at 35° and died after 3 days. The fungus was also grown on corn-meal agar at certain temperatures. At 30°, 25.5°, and 24.6° the mycelial colonies averaged 44.3, 40.4, and 40.2 mm. in diameter, respectively, in 4 days. The fact that this fungus made fair growth on corn-meal agar in 4 days at temperatures up to 30° is important, because Tucker (*8*) used the temperature responses of certain species of *Phytophthora* when grown on corn-meal agar at 26° to 28° for 4 days in his key for separating them.

LIFE HISTORY OF THE FUNGUS

Just how the red stem canker fungus attacks plants under natural conditions is not entirely clear. The natural assumption would be that infection takes place in the root or stem beneath the soil surface or near it and that the fungus then gradually progresses up the stem. In many instances, however, there is no evidence to support such an assumption. It appears that frequently infection must take place in the young growing tissues above ground, since there is no evident connection between the lesion and the underground parts of the plant.

An experiment was performed to learn whether infection could take place underground and, if so, whether the fungus could grow up the stem into the above-ground parts. Some soil was removed from one side of the base of young cowpea plants in 4 pots, a small piece of agar on which the red stem canker fungus was growing was placed against the underground part of the stem, and the soil was replaced. Two pots in which sterile agar was placed against the underground stems served as controls. In all, 22 plants were inoculated and 18 were held as controls. Only 4 of the inoculated plants and none of the controls became infected. In a later experiment performed in the same manner 60 percent of the plants inoculated were infected. Evidently, therefore, infection can take place below ground, although under the experimental conditions it took place much more readily above ground. When infection did take place underground, the fungus caused only a very narrow lesion that extended from the point of inoculation to a point well above the surface of the soil and then broadened out and involved a much greater portion of the stem (fig. 3).

The red stem canker fungus can penetrate the cortical cell walls as well as pass between them, especially in young tissue. The hyphae are greatly constricted where they pass through the cell wall. The walls of the affected cells as well as their densely granular contents are reddish. The presence of so much red in the cells imparts this color to the lesions.

When pieces of infected stem are placed in water, hyphae grow out into the water and sporangia are formed. In one instance many sporangia had formed in this manner in 16 hours and some had already germinated. This suggests that a layer of water on a lesion for 16 to 24 hours, and possibly less under conditions very favorable for the fungus, would suffice to enable the fungus to sporulate on the surface of a plant and reinfect it or an adjacent plant to which the zoospores might have been blown or on which they might have fallen.

TAXONOMY OF THE FUNGUS

A careful study of the germination of the sporangia of this cowpea fungus shows that it belongs in the genus *Phytophthora*. This fungus makes good growth on malt, corn-meal, and oatmeal agars after 6 days at 20° C., it produces oospores abundantly on these culture media, the antheridia are predominantly paragynous, and it makes a fair growth on corn-meal agar in 4 days at 26° to 28°. These facts place it in the species *P. cactorum* (Leb. and Cohn) Schroet. according to the key given by Tucker (*8*). The red stem canker fungus

grows at 27°, forms sexual bodies after being transferred from pea broth to distilled water as described by Leonian (*5, p. 29*), and has predominantly paragynous antheridia and papillate sporangia; hence it falls into the species *P. cactorum* in Leonian's key (*5*). The cowpea fungus differs from *P. cactorum* as given in Rosenbaum's key (*7*) only in that its oospores are smaller. The work of Tucker and Leonian, however, make it clear that rather wide variations in the size of the oospores are to be expected. Tucker (*8, p. 150*) gave a résumé of the measurements reported by various writers for oogonia and oospores of *P. cactorum*. Among the measurements given are those by Hartig (*3*), who stated that oogonia in *Fagus* sp. measured about 20μ. Measurements of oospores of *P. cactorum* given by other workers range from 15μ to 45μ.

The cowpea strain of *Phytophthora cactorum* differs from those studied by Leonian (*5*) in temperature response. Leonian stated that 31° C. seems to be the upper limit for the growth of this fungus and at that temperature growth is very poor. He reported that no strain grew at 32.5°. The cowpea fungus made a slight growth at 35°, but it died in 3 days at that temperature. At 31.5° it grew well. Tucker (*8*) considered 35° to be near the extreme limit for survival of *P. cactorum*. This temperature is also near the extreme or possibly a little above that for survival of the cowpea fungus. Tucker's strains of *P. cactorum* made some growth at 5° in 4 days. The strain from cowpea made no growth at 7° to 8° in 9 days. Beach's (*1*) strain of this fungus had a minimum temperature of 7° to 9°, which is about the same as that of the cowpea strain. Since the cowpea fungus varies in only a few minor respects from *P. cactorum* as conceived by most workers and the variations are within the range of variation described by other workers, there seems to be no reason why it should not be placed in this species.

No record of *Phytophthora cactorum* attacking cowpea was found in the literature. This fungus has been reported, however, as being parasitic on several other legumes as well as on a wide range of non-leguminous plants.

SUMMARY

A disease characterized by long, reddish lesions on the stems of cowpea plants and often resulting in the death of a part or all of the affected plant was found at Experiment, Ga. It is described and named red stem canker. A species of *Phytophthora* was isolated from the diseased stems and its pathogenicity was proved. Morphological and physiological studies showed that this cowpea *Phytophthora* falls within the range of the species *P. cactorum* as now conceived by most writers; hence it is considered to be a strain of that species.

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THE GENETICS OF MOSAIC RESISTANCE IN NICOTIANA GLUTINOSA[1]

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Nicotiana glutinosa L. has contributed to the development of varieties of tobacco (*N. tabacum* L.) that, from a practical standpoint, are immune to the tobacco mosaic virus. *N. glutinosa*, when inoculated with the tobacco mosaic virus, reacts with a necrotic spot at each point of entrance of the virus into the leaf, where the virus is usually localized.

Holmes[2] demonstrated that *N. glutinosa* carries a dominant factor pair *NN* for necrotic spotting, and he showed that the factor could be transferred to *N. tabacum*. The writer has developed many varieties of tobacco, including burley, dark air-cured, and dark-fired, that contain the *N* factor and remain completely free from mosaic in the field in spite of inoculation or growing next to affected susceptible plants. Some of these *NN* varieties (Ky 52, Ky 56, Ky 151, and Ky 160) are being grown commercially with complete success as to type of plant, quality of product, yield, and freedom from mosaic. The only injury from mosaic, under field conditions, has been when transplants were inoculated on the stalk with virus from living plants at pulling time, or when the midvein of a leaf was inoculated after rapid growth had commenced. Stalk inoculation resulted in a systemic necrotic disease that quickly killed the plant, while inoculation of the midvein sometimes resulted in a systemic necrotic disease that progressed more slowly but eventually caused death. Under ordinary farm conditions where susceptible varieties become heavily infected, the *NN* varieties show no injury from mosaic even though handled by men who chew or smoke natural leaf tobacco carrying the virus.

In attempting to understand the genetics of mosaic resistance in these varieties, questions arose as to (1) whether *N. glutinosa* would develop mottling in case of failure of the *N* factor to function, and (2) if mottling developed whether susceptibility is governed by a gene separate and distinct from the *N* gene. Or, to state the question another way, is the constitution of *N. glutinosa NN* or *NN AA* so far as its reaction to mosaic is concerned? To answer these questions a breeding experiment was set up in which *N. glutinosa* was crossed with a mosaic-resistant burley in which mosaic resistance had been derived from Ambalema, a mosaic-resistant variety from Colombia, South America. In Ambalema, and in the mosaic-resistant burley

[1] Received for publication April 17, 1948.

[2] HOLMES, F. O. INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO. Phytopathology. 28: 553–561, illus. 1938.

derived from it, mosaic resistance is governed by two recessive factor pairs a_1a_1 a_2a_2 that are apparently alleles of the factors for susceptibility A_1A_1 A_2A_2 contained in all mosaic-susceptible varieties of *N. tabacum*. If a mosaic-resistant plant carrying the recessive factors a_1a_1 a_2a_2 is crossed with *N. glutinosa* and the hybrid made fertile by doubling the chromosome number, any seedlings from this cross, or from a backcross with mosaic-resistant burley, that prove susceptible to tobacco mosaic must have received the susceptibility factor from *N. glutinosa*.

After the initiation of this work in June 1944 a partial answer to the first question was given in a paper by McKinney and Clayton [3] in which they showed that if, following inoculation with tobacco mosaic virus, a plant of *N. glutinosa* is kept at a temperature of 97° F., typical mosaic mottling will develop. It is evident, therefore, that *N. glutinosa* carries a factor for susceptibility to tobacco mosaic, but this did not answer the question as to whether the reaction to the *N* factor had been changed or whether the *N* factor had simply been temporarily put out of operation, thereby allowing a susceptibility factor of the *A* type to operate.

The hybrid *N. glutinosa* $NN \times$ mosaic-resistant burley a_1a_1 a_2a_2 was prepared. It proved to be sterile, as is usual with this interspecific hybrid. Lateral buds were treated with colchicine until finally a branch developed that was fertile. *N. digluta*,[4] as the new species is known, was used as the male parent in a backcross with the a_1a_1 a_2a_2 burley. Seed set in abundance but failed to germinate except for two seeds in a second sowing.[5]

One of the two seedlings of the cross *N. tabacum* a_1a_1 $a_2a_2 \times$ *N. digluta* NN a_1a_1 a_2a_2 was grown to maturity. It gave the necrotic reaction and so carried the *N* factor, and was partially fertile. Two hundred five F_2 seedlings were grown of which 24 gave the necrotic reaction and therefore carried the *N* factor, 60 were mosaic-susceptible and therefore were minus the *N* factor and carried at least one *A* or *A*-like factor for mottling, while 205 appeared to be nonnecrotic spotting but were mosaic-resistant and therefore did not carry either the *N* or *A*-like factors but only *a* factors.

The reciprocal cross *N. digluta* $\times$ *N. tabacum* produced viable seed. If *tabacum* chromosomes paired only with *tabacum*, and *glutinosa* only with *glutinosa*, all gametes of *N. digluta* should be 24+12 and the progeny of the cross *N. digluta* NN a_1a_1 $a_2a_2 \times$ *N. tabacum* a_1a_1 a_2a_2 should be (24+12)+24 and contain the *N* factor from *N. glutinosa*. All seedlings should develop necrotic spots when inoculated, and it should be necessary to grow the second generation of this cross to get the desired results. Actually, the seedlings were not uniform. Of 18

[3] McKinney, H. H., and Clayton, E. E. genotype and temperature in relation to symptoms caused in nicotiana by the mosaic virus. Jour. Hered. 36: 323–331, illus. 1945.

[4] Clausen, R. E. interspecific hybridization in nicotiana. VII. the cytology of hybrids of the synthetic species, digluta, with its parents, glutinosa and tabacum. Calif. Univ. Pubs. Bot. 11; [177]–211, illus. 1928.

[5] An examination showed that the endosperm had failed to develop in the majority of seeds, leaving a hollow shell. The reciprocal in which *N. digluta* was the female parent produced fertile seed. The chromosome number of the endosperm in the sterile cross was, theoretically, 24+24+36=84, while in the reciprocal cross that proved fertile it was 36+36+24=96 chromosomes.

plants grown to maturity and tested for their reaction to the tobacco mosaic virus, 17 were without the dominant *N* factor from *N. glutinosa*. Of the 17, 16 proved to be susceptible to tobacco mosaic virus, as they developed typical mottling, and therefore carried at least one *A* or *A*-like factor. One proved to be resistant to mosaic but did not develop necrotic spotting, indicating that it did not contain either the *N* or *A*-like factors but only the recessive *a* factors for resistance of the Ambalema type. All of the backcross plants were partially sterile, indicating their hybrid origin.

DISCUSSION AND CONCLUSIONS

The Ambalema type mosaic-resistant burley tobacco evidently does not contain any factors for mosaic susceptibility, as a single *A* factor for susceptibility has been demonstrated to be dominant over three *a* factors for resistance. *Nicotiana glutinosa* contains the *N* or necrotic spotting factor but when grown at 97° F. develops typical mosaic mottling. Therefore, the results of the present paper may be interpreted as proving that *N. glutinosa* contains not only the dominant *N* factor for necrotic spotting but also a dominant factor for susceptibility to mosaic. The temperature studies of McKinney and Clayton may be interpreted as showing that a temperature of 97° F. makes the *N* factor in *N. glutinosa* temporarily nonfunctional but allows the susceptibility factor to operate, with the result that *N. glutinosa* develops typical mosaic mottling. Mosaic-resistant tobacco varieties containing the *N* factor are essentially mosaic-susceptible varieties with the *N* or necrotic spotting factor probably substituted for the *N′* or *n′*[6] factor of *N. tabacum* but otherwise the genetic make-up seems to be identical with that of ordinary susceptible varieties of *N. tabacum*.

A variety of the genetic make-up $NN\ a_1a_1\ a_2a_2$ should be virtually immune to the tobacco mosaic virus because necrotic spots should develop so slowly that localization would be complete. This combination is still in the process of development.

[6] VALLEAU, W. D., and JOHNSON, E. M. AN OUTBREAK OF PLANTAGO VIRUS IN BURLEY TOBACCO. Phytopathology 33: 210–219, illus. 1943.

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